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Applied and Environmental Microbiology, Dec. 2010, p. 8110–8116 Vol. 76, No. 24
0099-2240/10/$12.00 doi:10.1128/AEM.01343-10

Associations between the Presence of Virulence Determinants and the Epidemiology and Ecology of Zoonotic *Escherichia coli*[▽]

K. M. O'Reilly,[1]† J. C. Low,[2] M. J. Denwood,[1] D. L. Gally,[3] J. Evans,[2] G. J. Gunn,[2] D. J. Mellor,[1] S. W. J. Reid,[1] and L. Matthews[1]*

Boyd Orr Centre for Population and Ecosystem Health, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G61 1QH, United Kingdom[1]; Scottish Agricultural College, Animal Health Group, Research Division, King's Buildings, West Mains Road, Edinburgh EH9 3JG, United Kingdom[2]; and Immunity and Infection Division, Roslin Institute and R(D)SVS, University of Edinburgh, Chancellor's Building, Edinburgh EH16 4SB, United Kingdom

Received 5 June 2010/Accepted 16 September 2010

The severity of human infection with pathogenic *Escherichia coli* depends on two major virulence determinants (*eae* and *stx*) that, respectively, produce intimin and Shiga toxin. In cattle, both may enhance colonization, but whether this increases fitness by enhancing cattle-to-cattle transmission in the field is unknown. In *E. coli* O157, the almost uniform presence of the virulence determinants in cattle isolates prevents comparative analysis. The availability to this study of extensive non-O157 *E. coli* data, with much greater diversity in carriage of virulence determinants, provides the opportunity to gain insight into their potential impact on transmission. Dynamic models were used to simulate expected prevalence distributions for serogroups O26 and O103. Transmission parameters were estimated by fitting model outputs to prevalence data from Scottish cattle using a Bayesian Markov chain Monte Carlo (MCMC) approach. Despite similar prevalence distributions for O26 and O103, their transmission dynamics were distinct. Serogroup O26 strains appear well adapted to the cattle host. The dynamics are characterized by a basic reproduction ratio (R_0) of >1 (allowing sustained cattle-to-cattle transmission), a relatively low transmission rate from environmental reservoirs, and substantial association with *eae* on transmission. The presence of stx_2 was associated with reduced transmission. In contrast, serogroup O103 appears better adapted to the noncattle environment, characterized by an R_0 value of <1 for plausible test sensitivities, a significantly higher transmission rate from noncattle sources than serogroup O26, and an absence of fitness benefits associated with the carriage of *eae*. Thus, the association of *eae* with enhanced transmission depends on the *E. coli* serogroup. Our results suggest that the capacity of *E. coli* strains to derive fitness benefits from virulence determinants influences the prevalence in the cattle population and the ecology and epidemiology of the host organism.

Human-pathogenic *Escherichia coli* may be categorized into different pathogen types that include enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) (26). The primary distinction is the ability of EHEC strains to produce Shiga toxin (Stx) due to the integration into the genome of a bacteriophage carrying the encoding gene (*stx*). Central to the pathogenesis of EPEC is its ability to colonize the intestinal mucosa by the formation of attaching and effacing (A/E) lesions (26). A key component of this complex adhesion system, which may be possessed by both EPEC and EHEC strains, is the production of intimin, encoded by the *eae* gene. The genes required for the formation of A/E lesions are carried on a 36-kb pathogenicity island termed the locus of enterocyte effacement (LEE) (24).

Cattle may carry high densities of these *E. coli* pathogen types, including those that cause serious human infections, such as *E. coli* O157. *E. coli* serogroups O26, O103, O145, and O111 have also emerged as increasingly important causes of human infection, and in some countries non-O157 serogroups now dominate *E. coli* serogroup O157 as a source of human infection (4, 16). These strains may often be asymptomatically carried by cattle (6, 10, 30, 37), yet intriguingly there is widespread carriage of virulence genes in cattle isolates of both O157 and non-O157 *E. coli* serogroups (13, 27).

Multilocus sequence typing (MLST) has shown that these *E. coli* pathogen types occur in many unrelated lineages and that EHEC strains are almost as closely related to EPEC strains as to other EHEC strains (41). The conclusion of these studies is that horizontally transmitted virulence genes may have been acquired independently on multiple occasions by EHEC and EPEC strains. As the horizontal acquisition of virulence is presumably under a selection pressure exerted by the host, driving bacterial mutation and recombination (41), the finding raises questions as to the function of these virulence determinants in the ecology and epidemiology of the strains in cattle.

Experimental work has shown that the virulence determinant *eae* can enhance colonization by *E. coli* O157 in ruminants (7, 8, 9, 31) and may increase shedding of the pathogen (7). It remains to be established whether this leads to enhanced transmission of the pathogen in the field. The role of Shiga toxin is less well understood. Historically, the lack of apparent clinical infection and pathology in cattle was attributed to a lack of vascular receptors for a critical toxin, Stx1 (33). However, recent work has shown the importance of Shiga toxin for adherence in culture systems and mice via nucleolin upregulation

* Corresponding author. Mailing address: Jarrett Building, School of Veterinary Medicine, University of Glasgow, Bearsden Road, Glasgow G61 1QH, United Kingdom. Phone: 44 (0)141 330 8175. Fax: 44 (0)141 330 5602. E-mail: louise.matthews@glasgow.ac.uk.

† Present address: Department of Infectious Disease Epidemiology, Faculty of Medicine, Imperial College, London, United Kingdom.

▽ Published ahead of print on 15 October 2010.

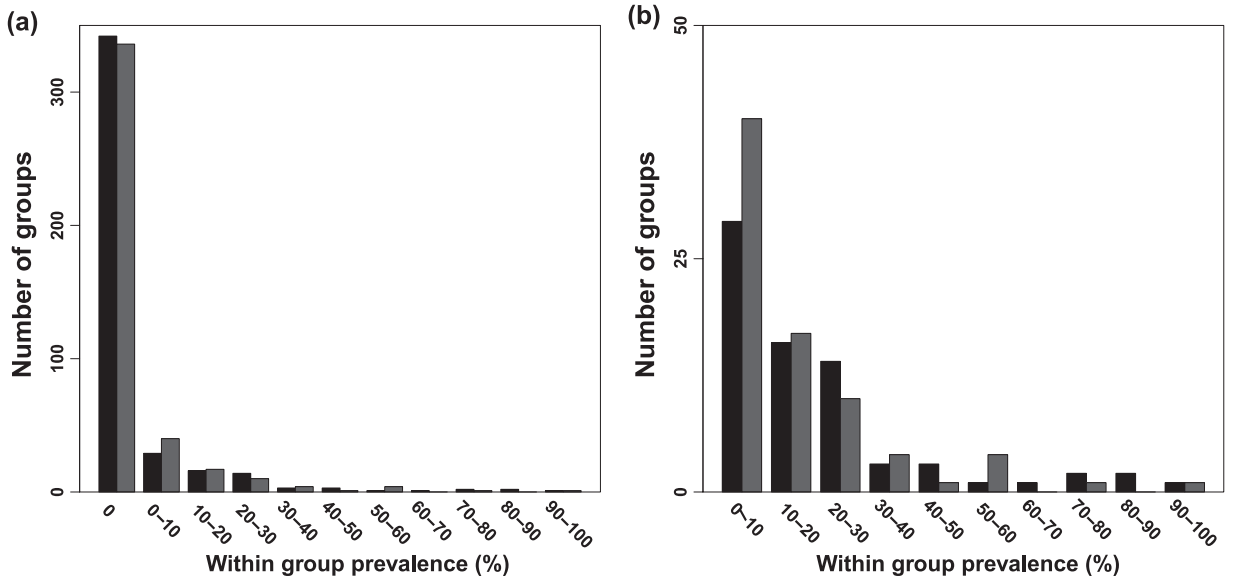

FIG. 1. Observed prevalence distributions for *E. coli* serogroups O26 (black) and O103 (gray) derived from a sample of 6,086 fecal pats from 414 cattle groups. (a) Full dataset; (b) dataset excluding observed zero prevalences, to expose the tails of the distributions.

and binding to intimin (35). In cattle, Stx2 has been demonstrated to enhance colonization of intestinal tissues (2, 3, 20), demonstrating for the first time a direct role of a cytotoxin in colonization of cattle. To date, the role of these virulence determinants has been assessed *in vitro* or within individual hosts, but as far as we are aware, their potential impact on transmission in the field has not been investigated.

A recent survey of the Scottish cattle population determined the prevalence and distribution of the *E. coli* serogroups O157, O26, and O103 and their virulence genes *eae* and *stx* (28). Serogroup O157 isolates were extremely uniform, almost all being stx_2 and *eae* positive (12), making it difficult to ascertain selective benefits to their presence. In contrast, the serogroup O26 and O103 isolates exhibited much greater diversity (28), which provides us with the opportunity to conduct comparative analyses of the transmission dynamics of strains with and without these key virulence determinants. Here, we use extensive cross-sectional data obtained from a prevalence survey of non-O157 *E. coli* (28) to quantify the transmission dynamics of the non-O157 strains.

The transmission of infection from individual to individual can be quantified in terms of the basic reproduction number, R_0, defined as the average number of secondary cases generated by a single infected individual in a naïve population (1). In large populations, if R_0 is >1, the infection on average will spread; if R_0 is <1, it is expected to decline. With small groups of individuals, it is recognized that stochastic or chance events will allow chains of infection to become extinct, requiring reintroduction for persistence of infection (17, 34).

Here, we quantify the transmission dynamics of the non-O157 strains in terms of the within-group R_0 and an immigration rate, λ, which captures transmission arising from movements of infected animals into the cattle management group or transmission from an environmental reservoir. We use the term environmental reservoir to refer to noncattle sources of infection that could also include other livestock or wildlife species. This allows us to quantify and contrast the mechanisms of persistence for serogroups O26 and O103 and to examine associations between the carriage of virulence genes and the strains' ecology and epidemiology. An improved understanding of the potential role of virulence determinants of human-pathogenic *E. coli* in the cattle reservoir may inform the prediction of the emergence of future virulent strains.

MATERIALS AND METHODS

Prevalence data. A national survey was carried out to determine the prevalence of *E. coli* serogroups O26, O103, O111, and O145 in feces of Scottish cattle between March 2002 and February 2004 (28). Only serogroups O26 and O103 were included in our analysis, as the prevalence of the other serogroups was either low (serogroup O145) or zero (serogroup O111). In total, 6,086 fresh fecal pats from 338 farms were tested. The 338 farms contained 414 groups of cattle that were managed separately. In each individual management group, sufficient fecal pats from the sampled farms were tested to ensure an 80% probability of identifying at least one positive pat when there was at least one shedding animal within the group. Of the 414 groups, O26 was isolated in 72 groups and O103 in 78. The observed cattle level prevalence was 4.1% for O26 and 2.8% for O103 (Fig. 1 shows the observed prevalence distributions). All isolates were additionally screened for the virulence genes encoding intimin (*eae*), Shiga toxin 1 (stx_1), and Shiga toxin 2 (stx_2) (28). In addition, a related virulence determinant that produces enterohemolysin (*ehxA*) and which is also associated with severe human disease was screened for, but as its presence was very closely correlated with the presence of *eae*, we did not consider it further (28). Table 1 shows the breakdown of the isolates by virulence gene carriage.

Strain definition. The presence of the virulence determinant *stx* was not significantly associated with the presence of *eae* (28). Within the *stx*-positive O26 groups, strains could be further classified as being positive for stx_1 and stx_2 or positive for stx_1 only. We found the percentage of stx_1-and-stx_2-positive isolates that were positive for *eae* (80.2%) not to be significantly different from the percentage of stx_1-only strains that were positive for *eae* (77.4%).

Our analyses investigated the transmission dynamics of the following strain combinations: (i) *eae*-positive or -negative strains, which are defined as those being positive/negative for *eae* irrespective of the *stx* presence; (ii) *stx*-positive or -negative strains, stx_1- and-stx_2-positive or -negative strains, and stx_1-only-positive or -negative strains, which are defined as those being positive/negative for the *stx* combination irrespective of the *eae* presence; and (iii) *eae*-positive and *stx*-positive or -negative strains. Note that the isolate numbers were too small to

TABLE 1. Numbers of O26 and O103 strains and frequency by virulence determinant

Strain	Virulence determinant			No. of strains
	stx_1	stx_2	*eae*	
O26	−	−	−	15
	+	−	−	18
	−	−	+	112
	+	+	−	7
	+	−	+	73
	+	+	+	24
Total (%)	122 (49.0)	31 (12.4)	209 (83.9)	249 (100)
O103	−	−	−	103
	+	−	−	1
	−	−	+	63
	+	+	+	1
Total (%)	2 (1.2)	1 (0.6)	64 (38.1)	168 (100)

consider any breakdown of the *eae*-negative strains into those which are *stx* positive or *stx* negative.

Test sensitivity. Reported test sensitivities for the identification of *E. coli* serogroups vary considerably in the literature (14, 29, 36, 38, 39). Estimates for the test sensitivity of the protocols used here found sensitivities ranging from 0.28 to 0.98 for pats inoculated with 10^2 to 10^6 CFU/g of O26 (14). Intermittent shedding has the potential to further reduce the sensitivity when used to establish prevalence in the field. Therefore, to assess the robustness of our results, all our analyses were conducted for test sensitivities from 0.10 to 1.0.

Mathematical modeling and parameter estimation. (i) Within-group dynamics. Dynamic transmission models were used to simulate the endemic prevalence and distribution of infection of the two serogroups across the population of livestock holdings. Following the modeling approach employed previously to describe *E. coli* O157 (21, 22, 23), within-farm transmission dynamics were described by a stochastic, individual-based susceptible-infected-susceptible model. Infections were assumed to arise in the susceptible population via two possible routes (Table 2): first, transmission from other infected individuals (with rate $\beta S/N$, where β is the transmission rate, S the number of susceptible individuals, and N the group size, giving $R_0 = \beta/\sigma$), and second, immigration of infection (at a rate of λS) from some external source, which could represent either the presence of an environmental reservoir or the movement onto the farm of an already infected individual. Here, we use the term environmental reservoir to refer to noncattle sources of infection that could also include other livestock or wildlife species. Infected individuals were assumed to recover (at a rate of σI) to the susceptible state.

The stochastic within-herd transmission dynamics can be described as follows in terms of the probabilities, p_i, of there being infected (i) animals in a group of N:

$$\frac{dp_i}{dt} = \sigma(i+1)p_{i+1} + \left(\lambda + \frac{\beta(i-1)}{N}\right)[N-(i-1)]\,p_{i-1} - \left[\sigma i + \left(\lambda + \frac{\beta i}{N}\right)(N-i)\right]p_i \quad \text{for } i = 1, N \tag{1}$$

$$p_0 = 1 - \sum_{r=1,N} p_r$$

Solving these equations gives the following expression for the equilibrium probabilities, p^{eq_i}.

$$p_{i+1}^{eq} = \frac{\left(\lambda + \beta\frac{i}{N}\right)(N-i)p_i^{eq}}{\sigma(i+1)} \tag{2}$$

i.e.,

$$p_{i+1}^{eq} = \frac{\left(\lambda_r + R_0\frac{i}{N}\right)(N-i)p_i^{eq}}{(i+1)} \tag{3}$$

TABLE 2. Transitions in stochastic susceptible-infected-susceptible model

Transition	Symbolic notation	Rate
Susceptible to infected	(S, I) to $(S-1, I+1)$	$\lambda S + \beta S/N$
Infected to susceptible	(S, I) to $(S+1, I-1)$	σI

where $R_0 = \beta/\sigma$ is the basic reproduction ratio and $\lambda_r = l/\sigma$ is the rate of immigration measured relative to the timescale for recovery.

(ii) Fitting the model to the data. A Bayesian Markov chain Monte Carlo (MCMC) approach was used to estimate the transmission parameters λ_r and R_0 by fitting the observed prevalence data to the predicted distribution described by equation 2. Specifically, the true number of positive animals in a given group was assumed to be distributed according to equation 3, and the observed number of positive isolates assumed to be a sample from a binomial distribution, Bin(N_S, Se · I/N), where I/N is the true proportion infected in the group, Se the assumed test sensitivity, and N_S the number of samples taken. Uniform priors for R_0 and λ_r in the ranges of [0, 2] and [0, 0.03], respectively, were selected, and a Metropolis Hastings algorithm (11, 15, 25) with a random proposal distribution was used to explore parameter space. A chain of 1,000,000 iterations was used to generate smooth posterior distributions for the parameters and minimize the Monte Carlo error. Parameter estimates were obtained, for a range of potential test sensitivities, for the full O26 and O103 datasets and for both serogroups with the data broken down by presence/absence of the virulence genes *eae* and *stx*.

RESULTS

Comparison of O26 and O103 transmission dynamics. Despite the broadly similar observed prevalence distributions (Fig. 1), the two serogroups possessed distinct transmission dynamics. For a test sensitivity of 0.5, the posterior distributions for the basic reproduction number, R_0, and the immigration rate, λ_r, measured relative to the recovery rate, were distinct. R_0 for O26 was significantly greater than that for O103, while the immigration rate for O26 was significantly less than that for O103 (Fig. 2a and b). These findings were confirmed for a range of assumed test sensitivities (from 0.1 to 1) (Fig. 2c and d).

For O26, the dynamics were characterized by an R_0 that exceeds 1, allowing sustained cattle-to-cattle transmission and a comparatively low immigration rate. For O103, the predicted R_0 was less than 1 over a wide range of test sensitivities, while the immigration rate was comparatively high.

Associations between the carriage of virulence determinants and cattle-to-cattle transmission. Transmission parameters were also estimated for *E. coli* O26 and O103 strains defined by the presence/absence of the virulence determinants *eae* and *stx*. The presence of *eae* was associated with substantially enhanced transmission of *E. coli* O26 (Fig. 3a), with strains positive for *eae* having R_0 estimates greater than 1 and strains negative for *eae* having R_0 estimates that were predominantly less than 1. In contrast, there was no association between the presence of *eae* and the transmission dynamics of *E. coli* O103 (Fig. 3b).

Overall, the presence of *stx* genes (either stx_1 only or both) had no association with the transmission of *E. coli* O26 (Fig. 3c, black lines), and there was no evidence of any interaction between the *stx*- and *eae*-possessing strains (Fig. 3c). However, when the *stx*-possessing strains were broken down into strains with either stx_1 only or stx_1 and stx_2 together, a difference in R_0 estimates was observed (Fig. 3d). To ascertain whether this difference in R_0 values between the stx_1-only or stx_1-and-stx_2 strains was significant, repeated sampling from the two poste-

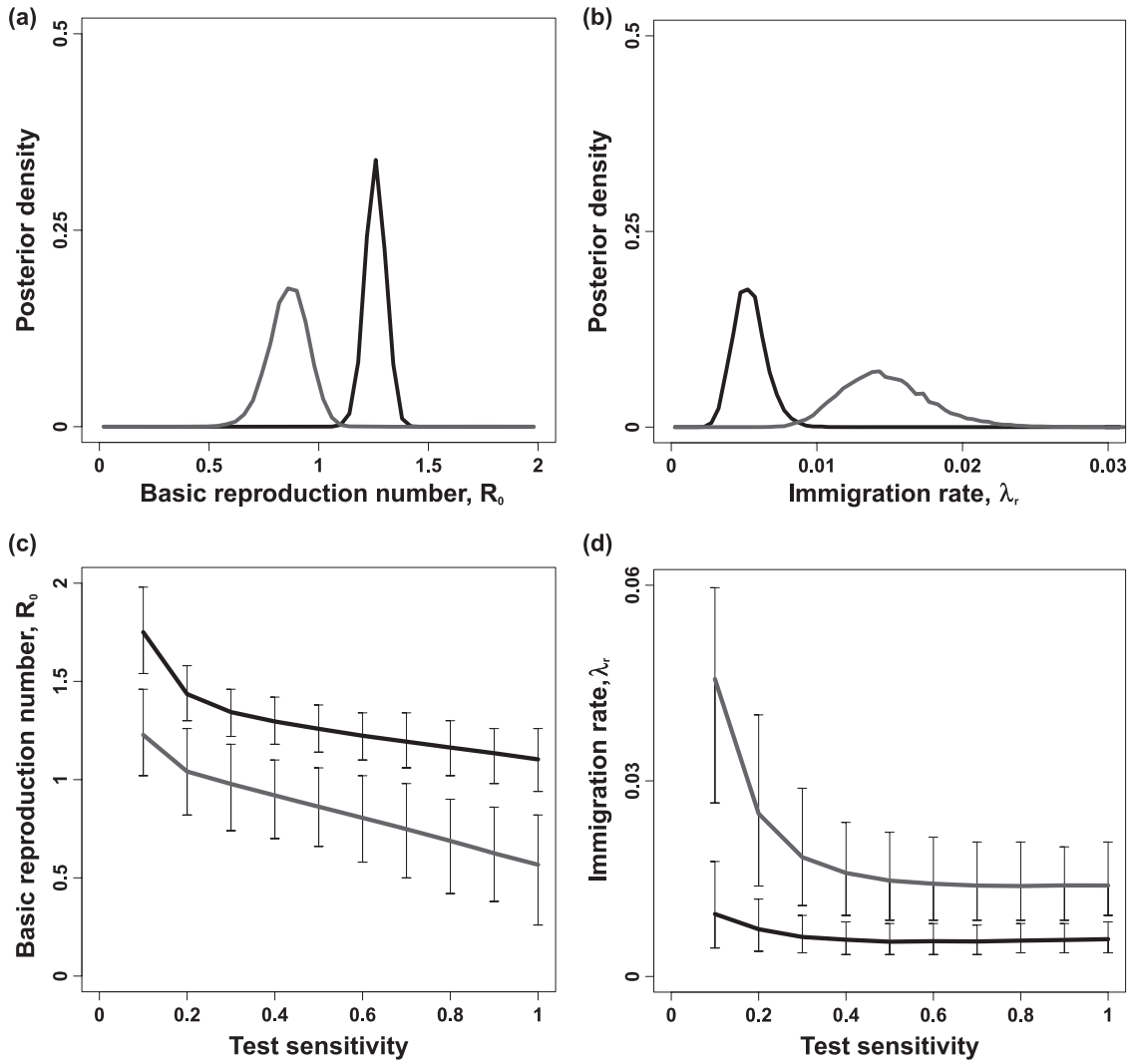

FIG. 2. Transmission dynamics of *E. coli* serogroups O26 (black) and O103 (gray). For a test sensitivity of 0.5, the posterior distributions for the basic reproduction ratio, R_0 (a), and the immigration rate, λ_r (b). For a range of assumed test sensitivities, estimates of the basic reproduction ratio, R_0 (c), and the immigration rate, λ_r (d). The error bars indicate 95% credible intervals.

rior distributions was used to generate 10,000 pairs of R_0 estimates for the two strains. Assuming a uniform prior on the test sensitivities and averaging across the range of test sensitivities, the overall probability of obtaining an R_0 estimate for the stx_1-and-stx_2 strains that was higher than an estimate for the stx_1-only strain was less than 2.3%. Thus, we conclude that stx_1-only strains have a slightly higher R_0 than strains possessing stx_1 and stx_2 ($P < 0.023$). Associations between the carriage of *stx* and transmission of O103 strains could not be examined due to its very low occurrence.

DISCUSSION

This study quantified the transmission dynamics in the Scottish cattle population of two major non-O157 *E. coli* serogroups and sought to identify associations between carriage of virulence determinants and their ecology and epidemiology. Strains were characterized by the presence of the virulence genes *eae* and *stx*, which are associated with severe disease in humans but whose role in the cattle reservoir is incompletely understood. Fitting dynamic models to the prevalence data for *E. coli* serogroups O26 and O103 revealed distinct transmission dynamics, reflecting different ecologies and epidemiologies of the serogroups. Specifically, analysis of the transmission dynamics revealed that, despite similar prevalence distributions, the two serogroups are maintained by different balances between R_0, the measure of cattle-to-cattle transmission, and the immigration rate, λ_r, which captures either transmission arising from movements of infected animals into the cattle management group or transmission from an environmental reservoir. Here, the term environmental reservoir refers to noncattle sources of infection that could also include other livestock or wildlife species.

E. coli serogroup O26 appears well adapted to the cattle host, with transmission dynamics characterized by an R_0 value of >1, allowing sustained cattle-to-cattle transmission, and a low transmission rate from an environmental reservoir relative

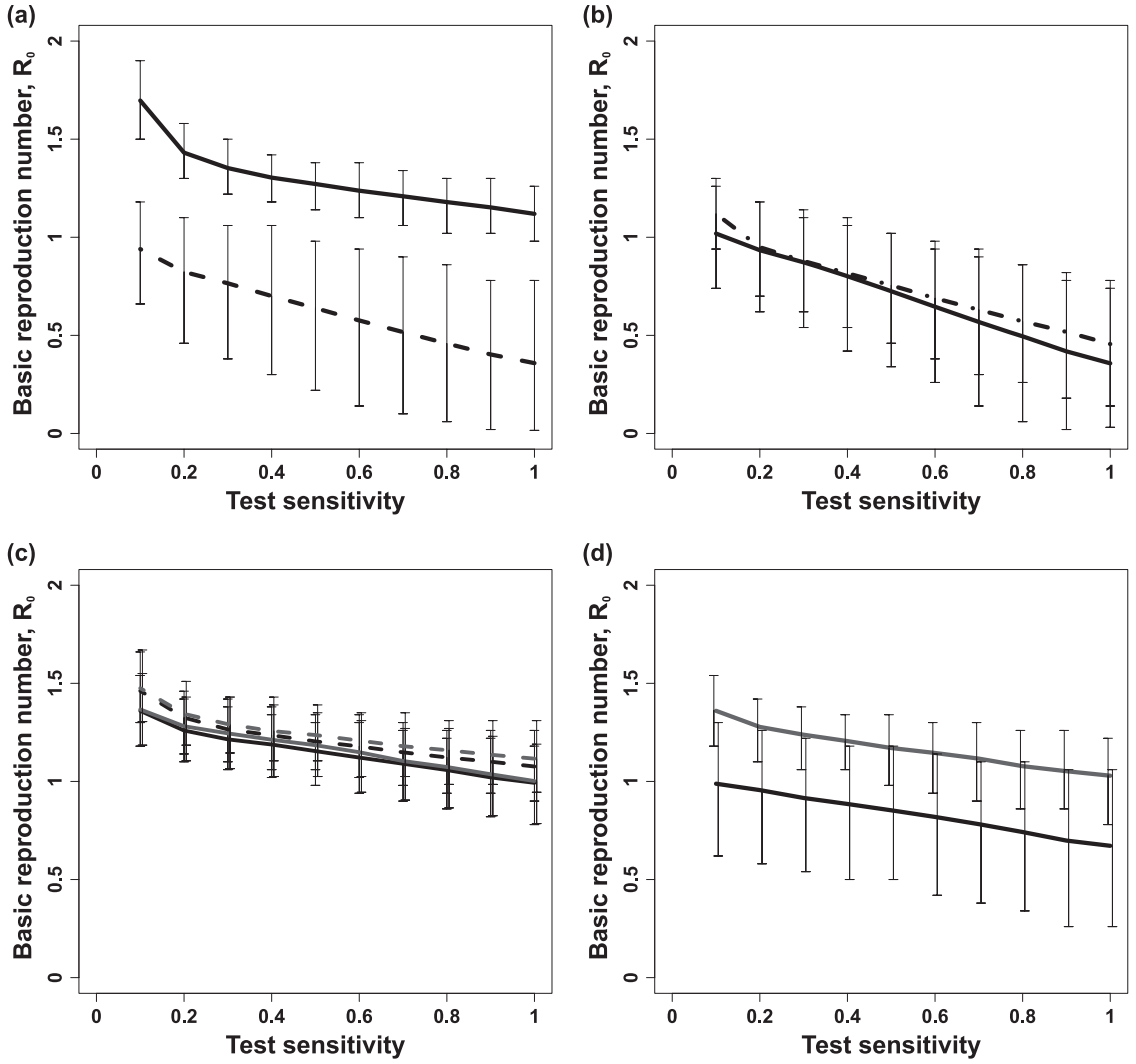

FIG. 3. Impact of virulence determinants on transmission. (a) R_0 for *E. coli* serogroup O26 *eae*-positive (solid) and *eae*-negative (dashed) strains. (b) R_0 for *E. coli* serogroup O103 *eae*-positive (solid) and *eae*-negative (dashed-dotted) strains. (c) R_0 for *E. coli* serogroup O26 *stx*-positive (solid black), *stx*-negative (dashed black), *stx*-positive-and-*eae*-positive (solid gray), and O26 *stx*-negative-and-*eae*-positive (dashed gray) strains. (d) R_0 for *E. coli* serogroup O26 stx_1-only-positive (gray) and O26 stx_1-and-stx_2-positive (black) strains. The error bars indicate 95% credible intervals and have been slightly displaced for clarity in panels c and d.

to *E. coli* O103. In contrast, serogroup O103 appears to be better adapted to the noncattle environment, with an R_0 value of <1 for plausible test sensitivities and a significantly higher environmental transmission rate than that of *E. coli* O26. Although our estimates for the immigration rate encompass both transmission from environmental reservoirs and introduction via infected animals moving into the management group, the lower prevalence of serogroup O103 relative to serogroup O26 strains in the national herd suggests that the differences in immigration rates arise from differences in transmission rates from environmental sources.

An alternative interpretation of these results leads similarly to the conclusion that O103 has improved survival in the noncattle environment. The alternative possibility is that apparent differences in immigration rates arise because our estimation procedure calculates the immigration rate relative to the timescale for recovery from infection, and these may differ between O103 and O26.

To examine this further, it is useful to consider the results of a previous comparative analysis of the transmission dynamics of serogroups O26 and O103 in calves (19). Liu et al. demonstrate no difference in the within-host periods of infection for O26 and O103. However, because transmission can occur via exposure to infected pats, the recovery period as defined in our model encompasses survival in the environment. Therefore, any differences in our recovery period would reflect differences in the short-term survival of infectivity. Thus, the observed differences in the immigration rate might therefore reflect a longer timescale for the decay of infectivity in the environment for O103. These two interpretations of the differences in immigration between serogroups lead to the conclusion that O103 has either improved survival or a greater presence in the noncattle environment.

The serogroups also differed in the proportion of isolates positive for *eae* (84% for *E. coli* O26 and 38% for *E. coli* O103)

and the extent to which *eae* was associated with enhanced transmission. The presence of *eae* was associated with a significant increase in the transmission of *E. coli* O26, corresponding to an increase in the basic reproduction ratio, R_0, from less than to more than 1. For *E. coli* O103, however, no association was observed. Our observation that the serogroup with the higher prevalence of *eae*-positive isolates corresponds to the serogroup with enhanced transmission of *eae*-positive strains suggests that the *eae* presence may translate into real selective advantages in the field.

That the association between the *eae* presence and transmission depends on the serogroup indicates a role for additional factors, such as virulence determinants not studied here, or the presence of different *eae* variants. Studies to characterize the *eae* allele in different serogroups have identified the *eae* allele for *E. coli* O26 as β1, while the *eae* allele for *E. coli* O103 is either θ or ε (5, 18, 32, 40). The different intimin types may be responsible for different host tissue tropisms (5), which may in turn contribute to enhanced transmission of some serogroups possessing specific alleles. Alternatively, as we did not measure the ability of strains from the two serogroups to form A/E lesions, there may be differences in the regulation of *eae* in the different genetic backgrounds.

Associations between the carriage of Shiga toxins and transmission could not be clearly elucidated by this study. Overall, the possession of *stx* genes (either stx_1 or both) had no association with the transmission of *E. coli* O26. However, breaking down the strains into stx_1-only and stx_1-and-stx_2 strains showed that the stx_1-only strains have a significant increase in the reproduction ratio, R_0, compared to the stx_1-and-stx_2 strains. As recent research has identified a potential role of stx_2 in colonization of the intestine (2, 3, 20), this result runs contrary to expectations and may indicate interactions between the strains arising, for example, from the presence of other virulence factors not examined by this study. We did not find any evidence of an interaction between the *stx*- and *eae*-possessing strains that might have been anticipated, given recent work that has shown the importance of Shiga toxin for adherence via binding to intimin (35). Associations between *stx* carriage and transmission were not assessed for *E. coli* O103 due to its very low occurrence (2 of 168 isolates), but this in itself may well be a consequence of an absence of any selective advantage in this serogroup.

Our results suggest the existence of strains with distinct modes of persistence. The serogroup O26 strains appear better adapted to the cattle host and able to derive fitness benefits from the virulence determinants responsible for severe human disease. In contrast, the serogroup O103 strains appear better adapted to the noncattle environment and lack the capacity for sustained cattle-to-cattle transmission. These strains may lack appropriate *eae* alleles or be missing other genetic determinants that allow the exploitation of *eae*. We recognize that this study has not considered the potential influence and role of other virulence factors possibly linked to specific serotypes. Our results suggest that the capacity to derive fitness benefits from virulence determinants influences their prevalence in the cattle population and the ecology and epidemiology of the host organism. By identifying the potential fitness benefits of these virulence determinants in the cattle host, our results may ultimately help inform the design of controls and predict emergence patterns for pathogenic human strains.

ACKNOWLEDGMENTS

We are grateful to the Scottish government, which funded a Centre of Excellence in Epidemiology, Population Health and Infectious Disease Control, and to the Foods Standards Agency (Scotland), which funded the data collection and analysis. L.M. is grateful to the Wellcome Trust for a Mathematical Biology Research Training Fellowship that funded the development of the quantitative approaches. The Scottish Agricultural College receives financial support from the Scottish Government's Rural and Environment Research Affairs Department (RERAD).

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